Spin Trapping of Reactive Oxygen Species by 1-hydroxy-3-carboxy-pyrrolidine (CP-H) in Biological Systems

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Introduction

Recent studies reported that 1-hydroxy-3-carboxy-pyrrolidine (CP-H) is highly sensitive for detection of reactive oxygen species (ROS) and qualified for quantification of ROS formation in biological systems. Therefore, we characterized CP-H and investigated its efficacy for measurement of ROS in biological systems using electron spin resonance.

Methods and Results

Characterizing CP-H in vitro with Krebs-Henseleit-buffer gassed with different oxygen concentrations revealed that there is high background oxidation (autoxidation) of CP-H which increased with rising metal ions concentration, temperature, pH, spin trap concentration and oxygen content and was reducible by metal chelators. Comparing hypoxic [pO_2~10mmHg] with normoxic [pO_2~150mmHg] conditions we found an increase from 16.2 ± 1.8 to 77.7 ± 4.2 [n=3, mean ± SEM] in signal intensity within 5 hours.

By stimulation of isolated buffer-perfused and ventilated rabbit lungs with 1µM Phorbol-12-Myristate-13-Acetate (PMA) signal intensity increased from 27.5 ± 6.5 by 21.1% ± 4.5% within 5 min, corresponding to an 8.6 ± 2.4-fold increase in the slope of CP-H oxidation [n=3]. Baseline autoxidation could be minimized from 74.8 ± 5.8 to 27.5 ± 6.7 [n=3] in signal intensity within 3 hours when lungs were perfused with 20µM deferoxamine and 5µM diethyldithiocarbamate.

Conclusion

We identified factors influencing and limiting autoxidation of CP-H. Using CP-H in isolated perfused lungs demonstrated that it is in principle suitable for ROS detection in biological systems, but has drawbacks because of its high autoxidation rate. Its specificity as an agent to detect oxygen radicals is limited by ROS-independent oxidative and reductive components occurring in biological systems.

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